

REVIEW ARTICLE

## The relationship between *Helicobacter pylori* and DNA hypermethylation in gastric cancer

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### ABSTRACT

Gastric cancer (GC) is one of the most common types of cancer across the world. GC carcinogenesis demonstrates a multi-step progression with the accumulation of genetic and epigenetic changes. The genetic changes on gene expression, without any change on deoxyribonucleic acid (DNA) sequencing, are named as "epigenetic". DNA methylation is the most commonly studied epigenetic modification in mammals. Hypermethylation is observed on CpG (—C—phosphate—G—) islands in GC, promoter regions of genes. This is a significant mechanism that causes functional losses in tumor suppressor genes. In gastric cancer, DNA hypermethylation is seen in many genes. *Helicobacter pylori* (*H. pylori*) are a type of spiral-shaped Gram-negative bacteria. It infects approximately half of the world's population. *H. pylori* is an important etiological factor that causes GC in human beings. Chronic *H. pylori* infection in human is associated with hypermethylation of promoter sequences of different genes. This paper provides a review of the current literature on the relationship between *H. pylori* infection and DNA hypermethylation in GC. *J Microbiol Infect Dis* 2015;5(4): 187-190

**Key words:** Gastric cancer, *Helicobacter pylori*, DNA hypermethylation.

### Mide kanserinde *Helicobacter pylori* ile DNA hipermetilasyonunun ilişkisi

#### ÖZET

Mide kanseri (MK) tüm dünyada sık görülen kanserlerden biridir. MK oluşumu genetik ve epigenetik değişikliklerin birikimi ile çok adımlı ilerleme gösterir. Deoksiribonükleik asit (DNA) dizisi değişmeden gen ifadesinde meydana gelen kalıtsal değişiklikler 'epigenetik' olarak adlandırılır. DNA metilasyonu memelilerde üzerinde en çok çalışılmış olan epigenetik modifikasyondur. Hipermetilasyon genlerin promotor bölgelerindeki CpG (—C—phosphate—G—) adacıklarında gözlenir. Bu, tümör baskılayıcı genlerde fonksiyon kaybına yol açan önemli bir mekanizmadır. MK'inde birçok gende DNA hipermetilasyonu görülür. *Helicobacter pylori* (*H. pylori*) spiral-şeklinde Gram negatif bir bakteridir. Dünya nüfusunun yaklaşık olarak yarısını enfekte eder. *H. pylori* insanlarda MK'ne yol açan önemli bir etyolojik faktördür. İnsanlarda kronik *H. pylori* enfeksiyonu farklı genlerin promotor bölge hipermetilasyonu ile ilişkilidir. Bu derlemede MK'indeki *H. pylori* enfeksiyonu ve DNA hipermetilasyonu arasındaki ilişkiye ait güncel literatür gözden geçirildi.

**Anahtar kelimeler:** Mide kanseri, *Helicobacter pylori*, DNA hipermetilasyonu.

#### INTRODUCTION

Gastric cancer (GC) holds the fourth rank with regard to world prevalence and the second rank with regard to cancer-related death in the world.<sup>1</sup> The prevalence of GC is associated with various factors, including geographic location, diet and genetic background of individuals. Ninety five percent of GCs are in the form of adenocarcinoma. According to Lauren's categorization, gastric adenocarcinomas are divided into two as intestinal and diffuse types by their microscopic configuration and growth pattern.<sup>2</sup> These types show different morphological,

clinical and epidemiological characteristics. This indicates that different pathways play a role in different histological types. Intestinal-type GCs are differential cancers and are considered to derive from gastric mucosa cells.<sup>3</sup> This type of GC is seen more commonly in elderly patients. In the etiology of intestinal-type GC, chronic gastritis, atrophy, intestinal metaplasia and *Helicobacter pylori* (*H.pylori*) infection play important role. The patients with high-grade atrophic gastritis and intestinal metaplasia have higher risks of developing GC.<sup>3</sup> Diffuse-type GC is undifferentiated and is more common among young people. It has poor prognosis. Compared to

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the intestinal-type GC, it is less associated with environmental factors.<sup>4</sup> The fact that diffuse-type GC is related with atrophic gastritis and intestinal metaplasia is an indication of poor prognosis.<sup>5</sup>

In recent years, early diagnosis and advancements in surgical techniques and perioperative care conditions have had positive effects on the clinical course of GC. However, GC still constitutes a major problem because of its high prevalence, poor prognosis and limited treatment options.<sup>6</sup> The rate of fatality in GC is higher than cancers such as breast and prostate. Because the disease is at an advanced stage when diagnosed, the rate of five-year survival is only 20-30%.<sup>5</sup> As it is the case in all cancers, GC carcinogenesis has a complex and multi-step progression. This paper provides a review of the latest literature on the relationship between *H. pylori* infection and DNA hypermethylation in GC.

### DNA methylation

Cancer is a disease caused by the activation of oncogenes and loss of function in tumor suppressor genes due to the accumulation of multiple genetic alterations and epigenetic modifications. Aberrant DNA methylation, commonly seen in cancer, is divided into two categories, i.e. genome-overall hypomethylation and regional hypermethylation. Global hypomethylation, discovered in 1980s, may be defined as the reduction of 5-methylcytosine content in the whole genome.<sup>7</sup> Genome-overall hypomethylation is seen in repetitive sequences such as Alu, LINE-1 and Sata, which constitute over 40% of the genome and are normally quite methylated. The genome-overall hypomethylation is seen almost in all cases of cancer and accelerates the progression of cancer by causing genomic instability.<sup>7</sup>

Genomic modifications that do not cause a change in DNA sequencing are defined as epigenetic. DNA methylation is the most frequently studied epigenetic mechanism. The regions rich in GC in the genome are named as CpG island, which is located in the promoter region in almost half of all genes. DNA methylation occurs with the addition of the methyl group to the fifth carbon of cytosine residues in CpG dinucleotide.<sup>8</sup> DNA methylation normally occurs in cellular events such as altered chromatin structure, X inactivation, differentiation and genomic imprinting.<sup>8</sup> The aberrant methylation of CpG islands in the promoter region causes transcriptional gene silencing. This is one of the major mechanisms that inactivate tumor suppressor genes.<sup>7</sup>

DNA methylation occurs with the enzymes named DNA methyltransferases (DNMT). There are different types of DNMT enzymes. DNMT1 ensures the methylation of the new DNA branch that develops in the course of DNA replication, and DNMT3a and DNMT3b facilitate de novo DNA methylation.<sup>8</sup> In general, the increase in methylation in the gene promoter region causes the reduction of gene expression. There are different mechanisms where methylation causes transcriptional suppression. One is that methylation directly prevents the binding of specific transcription factors to the transcriptional region.<sup>8</sup> The other one is methyl-CpG binding proteins (MBPs), which is one of the specific protein families that recognize methylated CpG. These enzymes interact with the specifically methylated DNA and mediate transcriptional suppression.<sup>8</sup>

The hypermethylation of the promoter region is the earliest and the most prevalent epigenetic modification in cancer.<sup>9</sup> There are some advantages of using the methylation in tumor as a marker. The methylation of specific CpG islands is more common than mutations in tumor. The degree of methylation may be evaluated quantitatively and the measurement is easy technically.<sup>10</sup> The DNA methylation pattern in tumor can potentially be used as a bioindicator of screening, prognosis and treatment in cancer. In GC, there are many genes known to be methylated. Among these genes are p14, p15, CDKN2A (p16), CDH1 (E-cadherin), hMLH1, GSTP1, CDH4, CDH1, APC, COX-2, TIMP-3, TSLC1 and RUNX3.<sup>11</sup>

### *Helicobacter pylori*

*H. pylori* was first discovered in 1983 by Marshall and Warren.<sup>12</sup> It exists in the stomach of over half of the world population. It is a spiral-shaped, micro-aerophilic, Gram-negative bacterium.<sup>7,13</sup> The prevalence of infection is over 20% in developed and over 90% in developing countries.<sup>14</sup> *H. pylori* infection mostly develops in early childhood. The infection continues if not cured.<sup>15</sup> In 1994, based on epidemiological evidence, the World Health Organization (WHO) included *H. pylori* in type I carcinogen category.<sup>7</sup>

In the animal model where Mongolian gerbils (*Meriones unguiculatus*) are used, chronic *H. pylori* infection rarely causes the development of GC. However, it apparently causes GC with mutagens such as N-methyl-N-nitrosourea (MNU) or N-methyl-N'-nitrosoguanidine.<sup>16,17</sup> This effect of *H. pylori* derives from its role in increasing chronic inflammation and cell reproduction. Cell proliferation increases the

development of more advanced motivation.<sup>17</sup> Pro-inflammatory mediators, produced excessively and continuously, are likely to contribute to the formation and development of tumors.<sup>18</sup>

Furthermore, *H. pylori* infection may lead to chronic inflammation due to the accumulation of reactive oxygen species (ROS) and oxidative DNA damage in gastric mucosa.<sup>8</sup> Various studies have shown that *H. pylori* is a multi-step etiological factor that first causes chronic gastritis and then gastric atrophy, intestinal metaplasia and dysplasia, ultimately leading to the development of GC.<sup>19</sup>

### ***H. pylori* and DNA methylation in GC**

The accumulation of genetic and epigenetic changes caused by chronic inflammation contributes to the development of cancer. Furthermore, many studies suggest that chronic inflammation is an important factor causing aberrant DNA methylation. This was supported by various non-cancerous tissue studies, including ulcerative colitis/colon, chronic hepatitis/liver, inflammatory reflux esophagitis/esophagus and chronic gastritis/stomach.<sup>17,20-22</sup>

In a study, the relationship between methylation in gastric mucosa and *H. pylori* was shown with CDH1 methylation.<sup>23</sup> CDH1 is a cell-cell adhesion glycoprotein, which is frequently inactivated by sporadic and germline mutations in sporadic/familial diffuse-type gastric cancers. It was reported in this study that CDH1 gene methylation is higher in *H. pylori*-positive gastric mucosa than in *H. pylori*-negative gastric mucosa.<sup>23</sup>

In gastritis caused by *H. pylori*, IFN-gama, IL-1-beta, TNF-alpha, NOS2 and COX2 genes associated with the inflammation were found to be highly expressed.<sup>24,25</sup> Studies with animals showed that the upregulation of IL-1-beta, NOS2 and TNF-alpha induced methylation.<sup>26,27</sup>

In non-cancerous inflammatory tissues, altered DNA methylation is evaluated as one of the early steps towards neoplastic change.<sup>28</sup> During the inflammation, the accumulation of DNA methylation contributes to the development of cancer. In non-cancerous gastric mucosa, the presence of genes such as THBD, LOX, HRASLS, FLNc and HAND1 in methylated form, though rarely, supports the supposition that some gastric carcinogens are likely to induce methylation. The most important gastric carcinogen is *H. pylori* infection, which increases the risk of GC by 2.2-21.<sup>11</sup> It was reported that the degree of methylation in gastric mucosa tissue is correlated with GC risk.<sup>17</sup> It was also stated that ab-

errant methylation was partially reversible after the eradication of *H. pylori*.<sup>29</sup>

The suppression of aberrant DNA methylation has been one of the new targets in the chemo-prevention of cancer. Various animal experiments were conducted for this purpose. However, the effectiveness of the suppression of aberrant methylation in cancers related to chronic inflammation could not be evaluated. In their study, Niwa et al. intended to prevent GC caused by *H. pylori* with the 5-aza-dC treatment in Mongolian gerbils model.<sup>30</sup> This is reported to be the first study to reduce the incidence of GC associated with chronic inflammation with the use of DNA demethylated agent.

### **CONCLUSION**

In cancer, methylation in tumor suppressor genes is the most common epigenetic mechanism. The methylation in tumor potentially constitutes the marker of the presence of *H. pylori* infection in the past. Further research on ambiguous points is of particular importance for the prevention, diagnosis and treatment of cancer and is likely to open up new horizons in cancer studies.

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